

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		ATTORNEY'S DOCKET NUMBER <b>1038-1153MIS:jb</b>
INTERNATIONAL APPLICATION NO. <b>PCT/CA99/01151</b>	INTERNATIONAL FILING DATE <b>December 2, 1999</b>	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR <b>09/857305</b> ) PRIORITY DATE CLAIMED <b>December 4, 1998</b>

## TITLE OF INVENTION

**TWO-STEP IMMUNIZATION PROCEDURE AGAINST CHLAMYDIA INFECTION**

## APPLICANT(S) FOR DO/EO/US

**Robert C. Brunham; and Andrew D. Murdin**

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1.  This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2.  This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3.  This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4.  The US has been elected by the expiration of 19 months from the priority date (Article 31).
5.  A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
  - a.  is attached hereto (required only if not communicated by the International Bureau).
  - b.  has been communicated by the International Bureau.
  - c.  is not required, as the application was filed in the United States Receiving Office (RO/US).
6.  An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
  - a.  is attached hereto.
  - b.  has been previously submitted under 35 U.S.C. 154(d)(4).
7.  Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
  - a.  are attached hereto (required only if not communicated by the International Bureau).
  - b.  have been communicated by the International Bureau.
  - c.  have not been made; however, the time limit for making such amendments has NOT expired.
  - d.  have not been made and will not be made.
8.  An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9.  An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)). - unsigned
10.  An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11.  A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12.  A copy of the International Search Report (PCT/ISA/210).

**Items 13 to 20 below concern document(s) or information included:**

13.  An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14.  An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15.  A **FIRST** preliminary amendment.
16.  A **SECOND** or **SUBSEQUENT** preliminary amendment.
17.  A substitute specification.
18.  A change of power of attorney and/or address letter.
19.  A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20.  A second copy of the published international application under 35 U.S.C. 154(d)(4).
21.  A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22.  Certificate of Mailing by Express Mail
23.  Other items or information:

**Initial Information Data Sheet**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR  
09/857305

INTERNATIONAL APPLICATION NO.

PCT/CA99/00151

ATTORNEY'S DOCKET NUMBER

1038-1153 MIS:jb

24. THE FOLLOWING FEES ARE SUBMITTED:

**BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :**

<input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO .....	\$1000.00
<input checked="" type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO .....	\$860.00
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO .....	\$710.00
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) .....	\$690.00
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) .....	\$100.00

**CALCULATIONS PTO USE ONLY****ENTER APPROPRIATE BASIC FEE AMOUNT =**

\$860.00

Surcharge of \$130.00 for furnishing the oath or declaration later than  20  30 months from the earliest claimed priority date (37 CFR 1.492 (e)).

\$0.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	40 - 20 =	20	x \$18.00	\$360.00
Independent claims	3 - 3 =	0	x \$80.00	\$0.00
Multiple Dependent Claims (check if applicable).			<input type="checkbox"/>	\$0.00

**TOTAL OF ABOVE CALCULATIONS =** \$1,220.00

<input type="checkbox"/> Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.	\$0.00
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**SUBTOTAL =** \$1,220.00

Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).	+ \$0.00
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**TOTAL NATIONAL FEE =** \$1,220.00

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable).	<input type="checkbox"/> \$0.00
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**TOTAL FEES ENCLOSED =** \$1,220.00

Amount to be: refunded	\$
charged	\$

- a.  A check in the amount of \$1,220.00 to cover the above fees is enclosed.
- b.  Please charge my Deposit Account No. \_\_\_\_\_ in the amount of \_\_\_\_\_ to cover the above fees. A duplicate copy of this sheet is enclosed.
- c.  The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. \_\_\_\_\_ A duplicate copy of this sheet is enclosed.
- d.  Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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24223

PATENT TRADEMARK OFFICE

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SIGNATURE

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NAME

24,973

REGISTRATION NUMBER

May 31, 2001

DATE

09/857305

JC05 Rec'd PCT/PTO 01 JUN 2001

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re National Phase of International Application

Applicant : Robert C. Brunham  
Appl'n. No. : PCT/CA99/01151  
Filed : December 2, 1999  
Title : TWO-STEP IMMUNIZATION PROCEDURE AGAINST CHLAMYDIA INFECTION  
Grp./A.U. :  
Examiner :  
Docket No. : 1038-1153 MIS:jb

May 31, 2001

**BY COURIER**

The Commissioner of Patents  
and Trademarks,  
Washington, D.C. 20231,  
U.S.A.

**PRELIMINARY MENDMENT**

Sir:

Please amend the above-identified application as follows:

**In the Specification:**

Before the first line of the specification, add the following;

“ **REFERENCE TO RELATED APPLICATION**

This application is a national phase application under 35 U.S.C. 371 of  
PCT/CA99/01151“

**REMARKS/ARGUMENTS**

The specification has been amended on page 1 to reflect that this application is a U.S. National Phase filing under 35 U.S.C. 371 of PCT/CA99/01151.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made.**"

It is believed that this application is now in condition for allowance and early and favourable consideration and allowance are respectfully solicited.

Respectfully submitted,

BY: Michael I. Stewart

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National Phase of PCT/CA99/01151

**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

In the Specification:

Before the first line of the specification, add the following;

**“REFERENCE TO RELATED APPLICATION”**

This application is a national phase application under 35 U.S.C. 371 of  
PCT/CA99/01151“

TITLE OF INVENTIONTWO-STEP IMMUNIZATION PROCEDURE  
AGAINST CHLAMYDIA INFECTIONFIELD OF INVENTION

5       The present invention relates to the field of immunology and, in particular, to a vaccination procedure for protection of a host against disease caused by infection with a bacterium of the Chlamydiaceae genus, particularly *Chlamydia trachomatis*.

BACKGROUND OF INVENTION

10      *Chlamydia trachomatis* is a species of the genus Chlamydiaceae, order Chlamydiales, *C. trachomatis* infects the epithelia of the conjunctivae and the genital tract, causing trachoma and a variety of sexually transmitted diseases (STDs) which can lead to, respectively, blindness or infertility. There are at least 15 serovars of *C. trachomatis*, of which A, B and C are causative agents of 15 trachoma, while serovars D, E, F, G, H, I, J and K are the most common causative agents of the Chlamydial STDs. *C. trachomatis* infections are endemic throughout the world. Trachoma is the leading cause of preventable blindness in developing nations, and it is estimated that 600 million people suffer from trachoma worldwide, with as many as 10 million of them being blinded by the disease. In 20 the United States, there are an estimated 3 million cases per year of STDs caused by *C. trachomatis*.

The pathogenesis of trachoma involves repeated ocular infections and the generation of a deleterious hypersensitivity response to chlamydial antigen(s) (refs. 1 to 4 - Throughout this specification, various references are referred to in 25 parenthesis to more fully describe the state of the art of which this invention pertains. Full bibliographic information for each citation is found at the end of the specification. The disclosure of these references are hereby incorporated by reference into the present disclosure). The available evidence supports the hypotheses that both secretory IgA and cell-mediated immune responses are 30 important components of protection. Ocular infection in a primate model induces rapid and persistent production of IgA in tears, whereas the presence of IgG in tears is transient, corresponding to the period of peak conjunctival inflammation

(refs. 5). Protective immunity following experimental ocular infection in a sub-human primate model is homotypic and resistance to ocular challenge correlates with the presence of serovar-specific antibodies in tears (refs. 1, 2, 6). Tears from infected humans neutralized the infectivity of homologous but not heterologous  
5 trachoma serovars for owl monkeys eyes (ref. 7) whereas passive humoral immunization with antitrachoma antibodies was not protective (ref. 8). Several lines of evidence indicate the importance of cell-mediated responses in protection from or clearance of chlamydial infection. B-cell deficient mice can resolve infection, whereas nude mice become persistently infected. Adoptive transfer of at  
10 least some chlamydia-specific T-cell lines or clones can cure persistently infected nude mice, and this anti-chlamydial activity is probably a function of the ability of the T-cells to secrete interferon- $\gamma$  (refs. 9 to 16).

Past attempts to develop whole-cell vaccines against trachoma have actually potentiated disease by sensitizing vaccinees (refs. 1, 2). Sensitization has  
15 been determined to be elicited to a 57 kD stress response protein (SRP)(HSP60) present in all serovars of *C. trachomatis*. Repeated exposure to the 57 kD SRP can result in a delayed hypersensitivity reaction, causing the chronic inflammation commonly associated with Chlamydial infections. Thus, an immunogenic preparation capable of inducing a strong and enduring mucosal neutralizing  
20 antibody response and a strong cellular immune response without sensitizing the host would be useful (ref. 17).

A most promising candidate antigen for the development of a vaccine is the chlamydial major outer membrane protein (MOMP) (refs. 18 to 20). Other surface proteins and the surface lipopolysaccharide are also immunogenic, but the  
25 antibodies they induce have not been found to be protective (refs. 21, 33). The MOMP, which is the predominant surface protein, is an integral membrane protein with a mass of about 40 kDa which, with the exception of four variable domains (VDs) designated I, II, III and IV, is highly conserved amongst serovars. The sequences of all four VDs have been determined for fifteen serovars (refs. 23,  
30 24). Antibodies capable of neutralizing chlamydial infectivity recognize the MOMP (refs. 25, 26, 27, 28). Epitopes to which MOMP-specific neutralizing monoclonal antibodies bind have been mapped for several serovars (refs. 21, 22,

- 29, 30, 31, 32, 33), and represent important targets for the development of synthetic or subunit vaccines. The binding sites are contiguous sequences of six to eight amino acids located within VDs I or II, and IV, depending on the serovar.
- Subunit immunogens (e.g. isolated MOMP or synthetic peptides) containing
- 5 MOMP epitopes can induce antibodies capable of recognizing intact chlamydiae (ref. 25). However, conventionally administered subunit immunogens are generally poor inducers of mucosal immunity. It would be useful to formulate chlamydial antigens in such a way as to enhance their immunogenicity and to elicit both humoral and cell-mediated immune responses.
- 10 Immune stimulating complexes (ISCOMs) are cage-like structures formed from a mixture of saponins (or saponin derivatives), cholesterol and unsaturated fatty acids. The components of ISCOMs are held together by hydrophobic interactions, and consequently proteins which are naturally hydrophobic (such as MOMP) or which have been treated to expose or add hydrophobic residues can be
- 15 efficiently incorporated into the ISCOMs as they form (refs. 34, 35, 36).

*C. trachomatis* naturally infects the mucosal surfaces of the eye and genital tract. Local antibody and local cellular immune responses are an important component of protection from mucosal infections. Consequently, it would be useful for a chlamydial vaccine to induce a mucosal immune response including

20 both cellular and antibody components.

DNA immunization is an approach for generating protective immunity against infectious diseases (ref. 37). Unlike protein or peptide based subunit vaccines, DNA immunization provides protective immunity through expression of foreign proteins by host cells, thus allowing the presentation of antigen to the

25 immune system in a manner more analogous to that which occurs during infection with viruses or intracellular pathogens (ref. 38). Although considerable interest has been generated by this technique, successful immunity has been most consistently induced by DNA immunization for viral diseases (ref. 39). Results have been more variable with non-viral pathogens which may reflect differences

30 in the nature of the pathogens, in the immunizing antigens chosen, and in the routes of immunization (ref. 40). Further development of DNA vaccination will depend on elucidating the underlying immunological mechanisms and broadening

its application to other infectious diseases for which existing strategies of vaccine development have failed.

The use of attenuated bacteria, in particular *S. typhimurium*, has recently been reported for delivery of plasmid DNA for genetic immunization (refs. 41, 5 42). This type of delivery offers the added benefit of delivering the DNA to cell types that induce a specific immune response, such as a mucosal immune response. This type of vaccination also offers the advantages of being safe, as many safe, attenuated strains of *Salmonella* are readily available, and cost effective.

10 EP 0192033 B1 and United States patent 5,770,714 describe the provision of a DNA construct for the expression, *in vitro*, of *Chlamydia trachomatis* MOMP polypeptides comprising the following operably linked elements:

a transcriptional promoter,  
a DNA molecule encoding a *C. trachomatis* MOMP polypeptide  
15 comprising a MOMP polynucleotide at least 27 base pairs in length from a sequence provided in Appendix A thereto, and

a transcriptional terminator, wherein at least one of the transcriptional regulatory elements is not derived from *Chlamydia trachomatis*.

There is no disclosure or suggestion in this prior art to effect DNA immunization  
20 with any such constructs.

Copending United States Patent Application No. 08/893,381 filed July 11, 1996 (WO 98/02546), assigned to University of Manitoba and the disclosure of which is incorporated herein by reference, describes an immunogenic composition for *in vivo* administration to a host for the generation in the host of a protective  
25 immune response to a major outer membrane protein (MOMP) of a strain of *Chlamydia*, comprising a non-replicating vector comprising a nucleotide sequence encoding a MOMP or MOMP fragment that generates a MOMP specific immune response, and a promoter sequence operatively coupled to the nucleotide sequence for expression of the MOMP or MOMP fragment in the host; and a  
30 pharmaceutically-acceptable carrier therefor.

Copending United States Patent Application No. 08/713,236 filed September 16, 1996 (WO 98/10789), assigned to Connaught Laboratories Limited

and the disclosure of which is incorporated herein by reference, describes an immunogenic composition, comprising the major outer membrane protein (MOMP) of a strain of *Chlamydia*, which may be *Chlamydia trachomatis*, and an immunostimulating complex (ISCOM).

5

### SUMMARY OF INVENTION

The present invention provides a novel immunization strategy to provide protection against disease caused by infection of members of the Chlamydiae family, particularly *Chlamydia trachomatis* and materials used therein. The immunization strategy provided herein leads to a stronger protective immune 10 response than other strategies.

According to one aspect of the invention, there is provided a method of immunizing a host against disease caused by infection by *Chlamydia* which comprises:

initially administering to the host an immunoeffective amount of an 15 attenuated bacteria harbouring a nucleic acid sequence encoding at least one immunoprotective-inducing *Chlamydia* protein or fragment thereof which generates a *Chlamydia* protein-specific immune response, operatively connected to a eukaryotic expression element, such as the cytomegalovirus promoter, and

subsequently administering to the host an immunoeffective amount of at 20 least one purified *Chlamydia* protein or fragment thereof which generates a *Chlamydia* protein specific immune response, of the same at least one *Chlamydia* protein or immunogenic fragment thereof as used in the initial administration, to achieve a *Chlamydia* specific protective immune response in the host.

The attenuated bacteria may be an attenuated strain of *Salmonella* or 25 *Shigella* and the nucleic acid sequence may be the MOMP gene or fragments thereof from a strain of *Chlamydia*, including *Chlamydia trachomatis* and *Chlamydia pneumoniae*. The boosting protein can be the MOMP protein or immunogenic fragments thereof from a strain of *Chlamydia*, including *Chlamydia trachomatis* and *Chlamydia pneumoniae*.

30 The administration steps may be effected to mucosal surfaces, such as by intranasal administration or by an initial intranasal administration of DNA followed by intramuscular administration of *Chlamydia* protein.

The immune response which is achieved in the host by the method of the invention preferably includes the production of *Chlamydia*-specific protection against live *Chlamydia* challenge and enhanced immunogenicity with greater delayed-type hypersensitivity (DTH) responses and high IgG<sub>2</sub> and IgG<sub>1</sub> antibody responses than achieved in other immunization procedures.

In another aspect, the present invention includes an attenuated strain of a bacterium harbouring a nucleic acid molecule encoding at least one immunoprotection-inducing *Chlamydia* protein or a fragment thereof which generates a *Chlamydia* protein specific immune response. The bacterium 10 preferably is a strain of *Salmonella*, such as a strain of *Salmonella typhimurium*. The invention extends to such attenuated strain of a bacterium when used as an immunogen and to the use of such attenuated strain in the manufacture of an immunogen for administration to a host.

The present invention, in a further aspect, provides a method of 15 immunizing a host against infection caused by a strain of *Chlamydia*, which comprises:

administering to the host an immunoeffective amount of an attenuated bacteria harbouring a nucleic acid molecule encoding at least one immunoprotection-inducing *Chlamydia* protein or a fragment thereof which 20 generates a *Chlamydia* protein specific immune response. Any of the embodiments described herein with respect to the priming administration in the prime-boost immunization protocol described herein applies to this aspect of the invention.

#### BRIEF DESCRIPTION OF DRAWINGS

25 Figure 1, containing panels A, B, C, D, E and F, shows the protection results of administering the MOMP-DNA either intramuscularly (panels A, B and C) or intranasally (panels D, E and F).

Figure 2, containing panels A, B and C, shows the protection results from mice immunized with *Salmonella* transfected with MOMP-DNA (pcDNA3).

30 Figure 3, containing panels A, B and C, shows the protection results from mice intranasally immunized with *Salmonella* transfected with pcDNA3, then boosted intramuscularly with MOMP embedded in ISCOM.

Figure 4, containing panels A, B and C, shows the DTH response (panel A) and the IgG<sub>2a</sub> (panel B) and IgG<sub>1</sub> (panel C) antibody responses from mice primed intranasally with the *Salmonella* delivered DNA (pcDNA3) then boosted intramuscularly with the MOMP-ISCOM protein. The data represent means ± SEM of log<sub>10</sub> titres of the antibody. \* represents p<0.05, when compared with naïve group and group immunized with 10<sup>8</sup> CFU pMOMP-*Salmonella* only.

Figure 5 shows the elements and construction of plasmid pcDNA3/MOMP, approximately 64 kb in size.

#### GENERAL DESCRIPTION OF THE INVENTION

The present invention relates to methods of immunization comprising an initial administration of a nucleic acid sequence encoding at least one *Chlamydia* protein or immunogenic fragment thereof, operatively connected to a eukaryotic expression element, delivered by an attenuated *Salmonella* and a subsequent administration of at least one protein or fragment thereof of the same protein of the *Chlamydia*. The at least one protein may comprise a *Chlamydia* protein, such as MOMP and may be formulated into an ISCOM for administration to the host. The at least one protein may be produced recombinantly or isolated from a chlamydial preparation.

To illustrate the present invention, plasmid DNA was constructed containing the MOMP gene and MOMP gene fragments from the *C. trachomatis* mouse pneumonitis strain (MoPn), which is a natural murine pathogen, permitting experimentation to be effected in mice. Primary infection in the model induces strong protective immunity to reinfection. For human immunization, a human pathogen strain is used, such as serovar C of *C. trachomatis*.

Any convenient plasmid vector may be used for the MOMP gene or fragment, such as pcDNA3, a eukaryotic expression vector (Invitrogen, San Diego, CA, USA), containing a suitable promoter, such as a cytomegalovirus promoter. The MOMP gene or MOMP gene fragment may be inserted in the vector in any convenient manner. The gene or gene fragments may be amplified from *Chlamydia trachomatis* genomic DNA by PCR using suitable primers and the PCR product cloned into the vector. The MOMP gene-carrying plasmid may be transferred, such as by electroporation, into *E. coli* for replication therein. A

MOMP-carrying plasmid, pcDNA3/MOMP, of approximately 64 kb in size, is shown in Figure 5. Plasmids may be extracted from the *E. coli* in any convenient manner.

The plasmid containing the MOMP gene or MOMP gene fragment may be 5 used to transform an attenuated *Salmonella* bacteria according to standard protocols, such as electroporation (ref. 43).

As described above, the primary (priming) immunization may be effected by administration of an attenuated bacterial vector, such as *Salmonella*, wherein the transfected DNA is not expressed in the bacterial vector. The expression of the 10 primary DNA is effected when the bacterial vector has released the DNA into the appropriate host cells, such as macrophages or dendritic cells. After uptake of the bacterial vector by the host cells, the auxotrophic bacteria dies after a few rounds of division due to their inability to synthesize the essential nutrients, such as amino acids or nucleotides. The plasmid DNA then is released into the cytoplasm 15 of the infected host cells and the encoded gene expressed in the host cell.

The boosting immunization may be a *Chlamydia* protein incorporated into a immunostimulatory complex (ISCOM) or a recombinantly produced *Chlamydia* protein. The *Chlamydia* protein can also be an isolated native *Chlamydia* protein, which is extracted from a *Chlamydia* extract.

20 It is clearly apparent to one skilled in the art, that the various embodiments of the present invention may have applications in the fields of vaccination and the treatment of *Chlamydia* infections. A further non-limiting discussion of such uses is further presented below.

#### EXAMPLES

25 The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or 30 render expedient. Although specific terms have been employed herein, such terms are intended as descriptive and not for purposes of limitation.

**Example 1:**

This Example illustrates the preparation of a plasmid vector containing the MOMP gene.

A pMOMP expression vector was made as described in the 5 aforementioned US Patent Application No. 08/893,381 (WO 98/02546). Briefly, the MOMP gene was amplified from *Chlamydia trachomatis* mouse pneumonitis (MoPn) strain genomic DNA by polymerase chain reaction (PCR) with a 5' primer (GGGGATCCGCCACCATGCTGCCTGTGGGAATCCT) (SEQ ID NO:1) which includes a BamH<sub>I</sub> site, a ribosomal binding site, an initiation codon and the 10 N-terminal sequence of the mature MOMP of MoPn and a 3' primer (GGGGCTCGAGCTATTAAACGGAAC TGAGC) (SEQ ID NO:2) which includes the C-terminal sequence of the MoPn MOMP, Xhol site and a stop codon. The DNA sequence of the MOMP leader peptide gene sequence was excluded. After digestion with BamH<sub>I</sub> and Xhol, the PCR product was cloned into the pcDNA3 15 eukaryotic II-selectable expression vector (Invitrogen, San Diego) with transcription under control of the human cytomegalovirus major intermediate early enhancer region (CMV promoter). The MOMP gene-encoding plasmid was transferred by electroporation into *E. coli* DH5 $\alpha$ F which was grown in LB broth containing 100  $\mu$ g/ml of ampicillin. The plasmids were extracted by Wizard<sup>TM</sup> Plus 20 Maxiprep DNA purification system (Promega, Madison). The sequence of the recombinant MOMP gene was verified by PCR direct sequence analysis, as described (ref. 44). Purified plasmid DNA was dissolved in saline at a concentration of 1 mg/ml. The DNA concentration was determined by DU-62 25 spectrophotometer (Beckman, Fullerton, CA) at 260 nm and the size of the plasmid was compared with DNA standards in ethidium bromide-stained agarose gel.

The MOMP gene containing plasmid, pcDNA3/MOMP, and its constitutive elements are shown in Figure 5.

**Example 2:**

30 This Example illustrates DNA immunization of mice.

A model of murine pneumonia induced by the *C. trachomatis* mouse pneumonitis strain (MoPn) was used (ref. 45). Unlike most strains of *C.*

*trachomatis*, which are restricted to producing infection and disease in humans, MoPn is a natural murine pathogen. It has previously been demonstrated that primary infection in this model induces strong protective immunity to reinfection. In addition, clearance of infection is related to CD4 Th1 lymphocyte responses 5 and is dependent on MHC class II antigen presentation (ref. 45).

Three different concentrations of MOMP-DNA were compared, administered either intramuscularly or intranasally (Figure 1). The results clearly show that mucosal delivery of naked MOMP-DNA is protective and appeared more so than intramuscularly delivered MOMP-DNA. Intranasal delivery of 10 MOMP-DNA was evaluated in multiple experiments to determine its reproducibility. As shown in Table 1, mucosal delivery of MOMP-DNA evoked protective immune responses but the magnitude of the protective index was highly variable, ranging from 0.5 to 4.1 log<sub>10</sub> protection in different experiments. The basis for such variability may be due to the limited immunogenicity of naked 15 DNA vaccination since challenging vaccinated animals with a higher inoculum of MoPn markedly reduced the protective index. Naked DNA applied to a mucosal surface may also have a very variable fate with some being degraded by extracellular nucleases and some being taken up the somatic cells.

**Example 3:**

20 This Example illustrates the delivery of DNA with attenuated *Salmonella*. *Salmonella typhimurium* strain 22-4 is described in ref. 46. Such strain was transfected with pcDNA3/MOMP and pcDNA3 by electroporation. Attenuated strains of *Salmonella*, transfected with plasmid DNA, were cultured for 16 to 25 hours at 37°C, without shaking in Luria Broth (LB) medium containing 100 µg/ml 25 ampicillin. Bacteria were collected by centrifugation and resuspended in PBS. Different concentrations of *Salmonella* were diluted with PBS and the same volume of 10% sodium bicarbonate was added immediately before immunization. Groups of 5 to 10 female Balb/c mice, 6 to 8 weeks of age, were deprived of water 30 for 5 to 6 hours before immunization. Approximately 10<sup>5</sup> to 10<sup>10</sup> CFU of bacteria in 100 µl were fed by feeding needles (Ejay International Inc.). Four inoculations at 2 week intervals were administered.

As shown in Figure 2, mice immunized with *Salmonella* transfected with MOMP-DNA had partial protection against lung challenge with MoPn. Immunization at one mucosal surface (the gut) provides protection against challenge infection at a distant mucosal surface (the lung).

5    **Example 4:**

This Example illustrates a DNA prime and protein boost immunization schedule in mice.

MOMP-DNA transfected *Salmonella*, prepared as described in Example 3, administered at  $10^8$  cfu was compared to MOMP-DNA transfected *Salmonella* administered at  $10^6$  cfu among groups of Balb/c mice orally immunized at two-week intervals on four occasions. Mice immunized with  $10^6$  cfu had a single protein boost intramuscularly with 1 µg MoPn MOMP embedded in ISCOM (14) at the time of the fourth immunization. The ISCOM preparation was prepared as described in aforementioned US Patent Application No. 08/718,236 (WO98/10789). The mice were challenged with 5000 IFU MoPn EB intranasally two weeks after the last immunization. Challenged mice were sacrificed at day 10 postinfection. The body weight was measured daily after infection until mice were sacrificed (Figure 3, panel A). These mice were much better protected than mice given  $10^8$  cfu *Salmonella* without a protein boost, as described in Example 3. *Chlamydia* EB growth in the lungs at day 10 postinfection was analyzed by quantitative tissue culture (Figure 3, panel B and C). In Figure 3, panel B, the data represents the mean  $\pm$  SEM of  $\log_{10}$  IFU per lung of 5 to 6 mice and panel C represents the results observed in individual mice. DNA primed, protein boosted mice also demonstrated enhanced immunogenicity with greater DTH responses (Figure 4, panel A) and higher serum IgG<sub>2</sub> and IgG<sub>1</sub> antibody responses (Figure 4, panels B and C). Sera were collected from immunized mice 2 weeks after the last immunization. MoPn-specific IgG<sub>2a</sub> (panel B) and IgG<sub>1</sub> (panel C) antibodies were tested by ELISA.

20    **Example 5:**

30    This Example describes the measurement of MoPn-specific delayed-type hypersensitivity (DTH).

To evaluate DTH, 25  $\mu$ l of ultraviolet (UV)-killed MoPn EBs ( $2 \times 10^5$  IFU) in SPG buffer 25 was injected into the right hind footpad of mice and the same volume of SPG buffer was injected into the left hind footpad as a control. Footpad swelling was measured at 48 hours and 72 hours post injection using a dila-gauge 5 caliper. The difference between the thickness of the two footpads was used as a measure of the DTH response.

#### SUMMARY OF DISCLOSURE

In summary of this disclosure, the present invention provides methods of immunizing a host against *Chlamydia* infection using DNA carried by an 10 attenuated bacteria and materials used in such procedures. Modifications are possible within the scope of the invention.

**Table 1** Intranasal (IN) immunization with MOMP-DNA evokes protective immunity to *Chlamydia trachomatis* MoPn lung infection.

EXPERIMENT Number	LOG10 IFU/LUNG		PROTECTIVE Index	CHALLENGE Inoculum (IFU)
	PcDNA3-IN	pMOMP-IN		
2	4.93 ± 0.68 (N=7)	3.65 ± 0.94 (N=6)	1.28	1000
3	6.1 ± 0.32 (N=4)	3.0 ± 1.15 (N=4)	4.1	5000
4	4.4 ± 0.32 (N=7)	3.9 ± 0.13 (N=7)	0.5	5000 X 2
7	5.39 ± 0.3 (N=8)	3.8 ± 0.63 (N=8)	1.59	5000

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CLAIMS

What we claim is:

1. A method of immunizing a host against infection caused by a strain of *Chlamydia*, which comprises:

initially administering to the host an immunoeffective amount of an attenuated bacteria harbouring a nucleic acid molecule encoding at least one immunoprotection-inducing *Chlamydia* protein or a fragment thereof which generates a *Chlamydia* protein specific immune response, and

subsequently administering to the host an immunoeffective amount of at least one purified *Chlamydia* protein or a fragment thereof which generates a *Chlamydia* protein specific immune response, of the same at least one *Chlamydia* protein as the initial administration to achieve a *Chlamydia* specific protective immune response in the host.

2. The method of claim 1 wherein said immunoprotection inducing *Chlamydia* protein or fragment thereof is a major outer membrane protein (MOMP) of a strain of *Chlamydia*.
3. The method of claim 2 wherein said strain of *Chlamydia* is a strain of *Chlamydia pneumoniae*.
4. The method of claim 2 wherein said strain of *Chlamydia* is a strain of *Chlamydia trachomatis*.
5. The method of claim 1 wherein said nucleic acid molecule is provided in a vector comprising the same and a promoter sequence operatively coupled to said nucleic acid molecule for expression of said *Chlamydia* protein or fragment thereof in said host.
6. The method of claim 5 wherein said nucleic acid molecule encodes a full-length major outer membrane protein (MOMP) of a strain of *Chlamydia*.
7. The method of claim 6 wherein said strain of *Chlamydia* is a strain of *Chlamydia pneumoniae*.
8. The method of claim 6 wherein said strain of *Chlamydia* is a strain of *Chlamydia trachomatis*.
9. The method of claim 1 wherein said attenuated bacteria is an attenuated strain of *Salmonella*.

10. The method of claim 5 wherein said promoter is a cytomegalovirus promoter.
11. The method of claim 5 wherein said vector is a plasmid vector.
12. The method of claim 11 wherein said plasmid vector has the identifying characteristics of pcDNA3/MOMP as seen in Figure 5.
13. The method of claim 1 wherein said immunoprotection-inducing chlamydial protein used in said subsequent administration step is administered incorporated into an immunostimulating complex (ISCOM).
14. The method of claim 13 wherein said chlamydial protein or fragment thereof is a major outer membrane protein (MOMP) of a strain of *Chlamydia*.
15. The method of claim 14 wherein said strain of *Chlamydia* is a strain of *Chlamydia pneumoniae*.
16. The method of claim 14 wherein said strain of *Chlamydia* is a strain of *Chlamydia trachomatis*.
17. The method of claim 1 wherein said first administration step is effected to mucosal surfaces.
18. The method of claim 17 wherein said first administration step is effected by intranasal administration and said second administration step is effected by intramuscular administration.
19. An attenuated strain of a bacterium harbouring a nucleic acid molecule encoding at least one immunoprotection-inducing *Chlamydia* protein or a fragment thereof which generates a *Chlamydia* protein specific immune response.
20. The attenuated strain of claim 19 wherein said immunoprotection inducing *Chlamydia* protein or fragment thereof is a major outer membrane protein (MOMP) of a strain of *Chlamydia*.
21. The attenuated strain of claim 20 wherein said strain of *Chlamydia* is a strain of *Chlamydia pneumoniae*.
22. The attenuated strain of claim 20 wherein said strain of *Chlamydia* is a strain of *Chlamydia trachomatis*.
23. The attenuated strain of claim 19 wherein said nucleic acid molecule is provided in a vector comprising the same and a promoter sequence operatively

coupled to said nucleic acid molecule for expression of said *Chlamydia* protein or fragment thereof in said host.

24. The attenuated strain of claim 23 wherein said promoter is a cytomegalovirus promoter.

25. The attenuated strain of claim 23 wherein said vector is a plasmid vector.

26. The attenuated strain of claim 25 wherein said plasmid vector has the identifying characteristics of pcDNA3/MOMP as seen in Figure 5.

27. The attenuated strain of claim 19 wherein said attenuated bacteria is an attenuated strain of *Salmonella*.

28. The attenuated strain of claim 27 wherein said attenuated strain of *Salmonella* is an attenuated strain of *Salmonella typhimurium*.

29. A method of immunizing a host against infection caused by a strain of *Chlamydia*, which comprises:

administering to the host an immunoeffective amount of an attenuated bacteria harbouring a nucleic acid molecule encoding at least one immunoprotection-inducing *Chlamydia* protein or a fragment thereof which generates a *Chlamydia* protein specific immune response.

30. The method of claim 29 wherein said immunoprotection inducing *Chlamydia* protein or fragment thereof is a major outer membrane protein (MOMP) of a strain of *Chlamydia*.

31. The method of claim 30 wherein said strain of *Chlamydia* is a strain of *Chlamydia pneumoniae*.

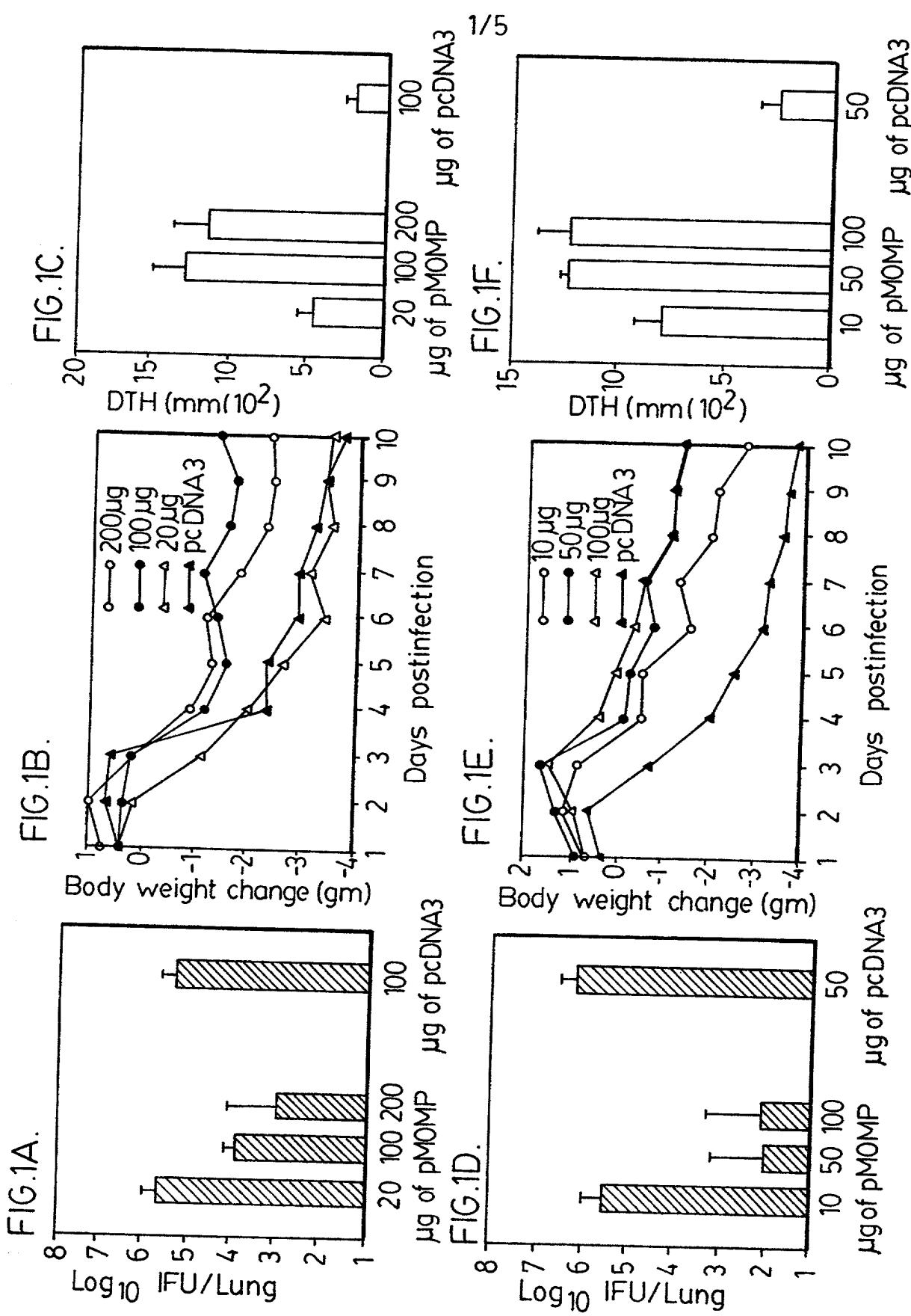
32. The method of claim 30 wherein said strain of *Chlamydia* is a strain of *Chlamydia trachomatis*.

33. The method of claim 29 wherein said nucleic acid molecule is provided in a vector comprising the same and a promoter sequence operatively coupled to said nucleic acid molecule for expression of said *Chlamydia* protein or fragment thereof in said host.

34. The method of claim 33 wherein said promoter is a cytomegalovirus promoter.

35. The method of claim 33 wherein said vector is a plasmid vector.

36. The method of claim 35 wherein said plasmid vector has the identifying characteristics of pcDNA3/MOMP as seen in Figure 5.
37. The method of claim 29 wherein said attenuated bacteria is an attenuated strain of *Salmonella*.
38. The method of claim 37 wherein said attenuated strain of *Salmonella* is an attenuated strain of *Salmonella typhimurium*.
39. The method of claim 29 wherein said administration is effected to mucosal surfaces.
40. The method of claim 39 wherein said administration is effected by intranasal administration.



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FIG.2A.

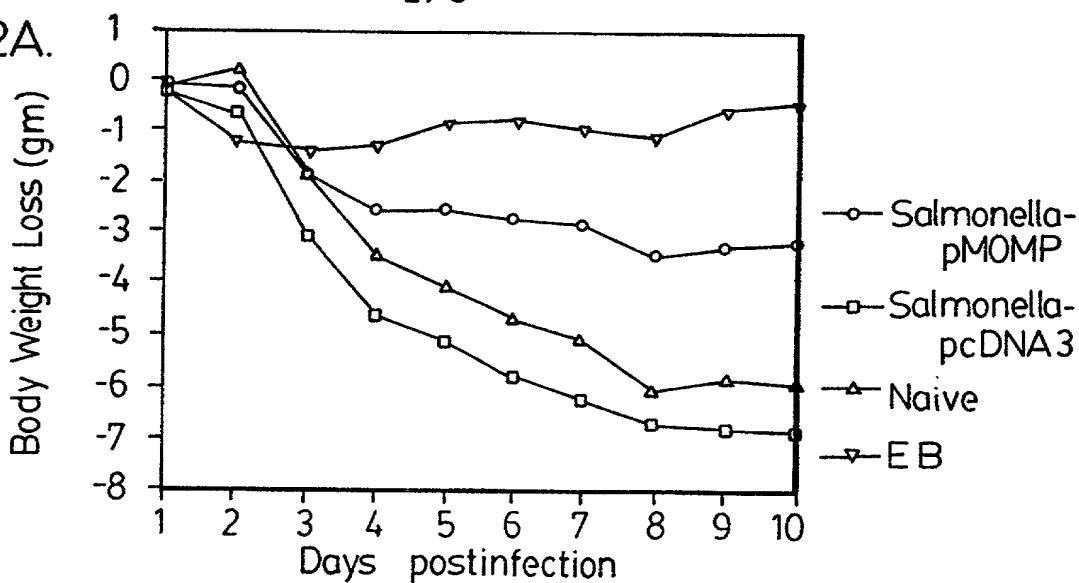


FIG.2B.

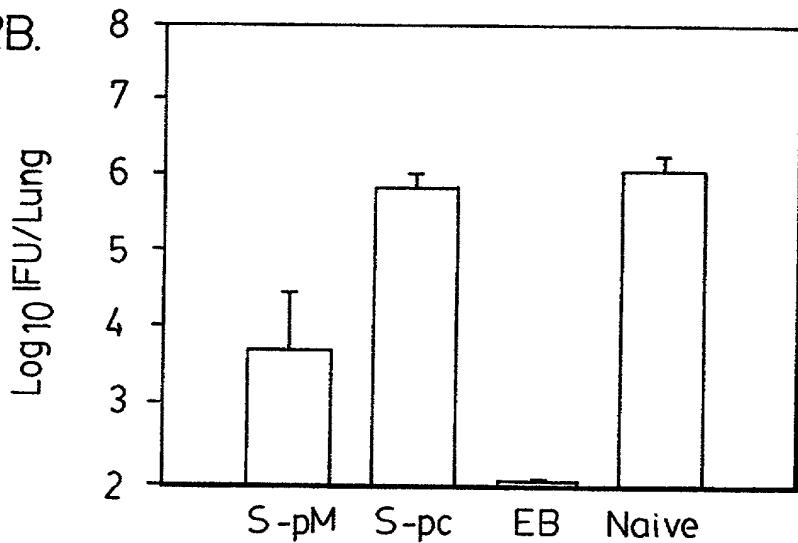


FIG.2C.

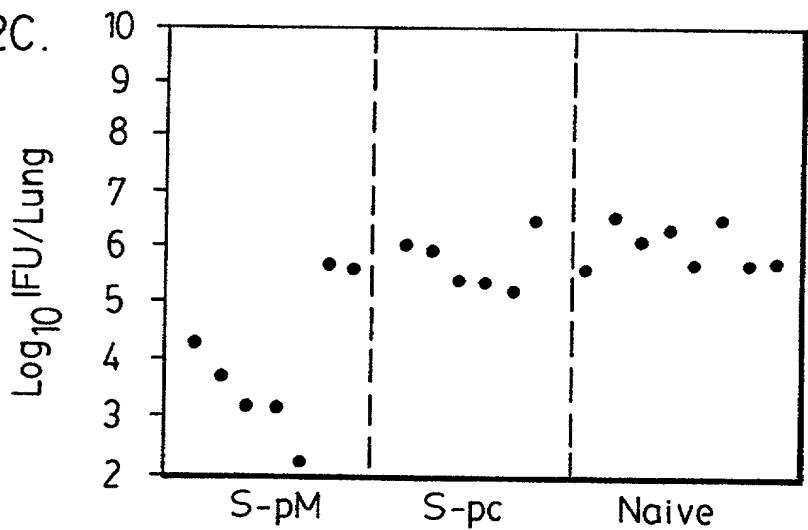


FIG.3A.

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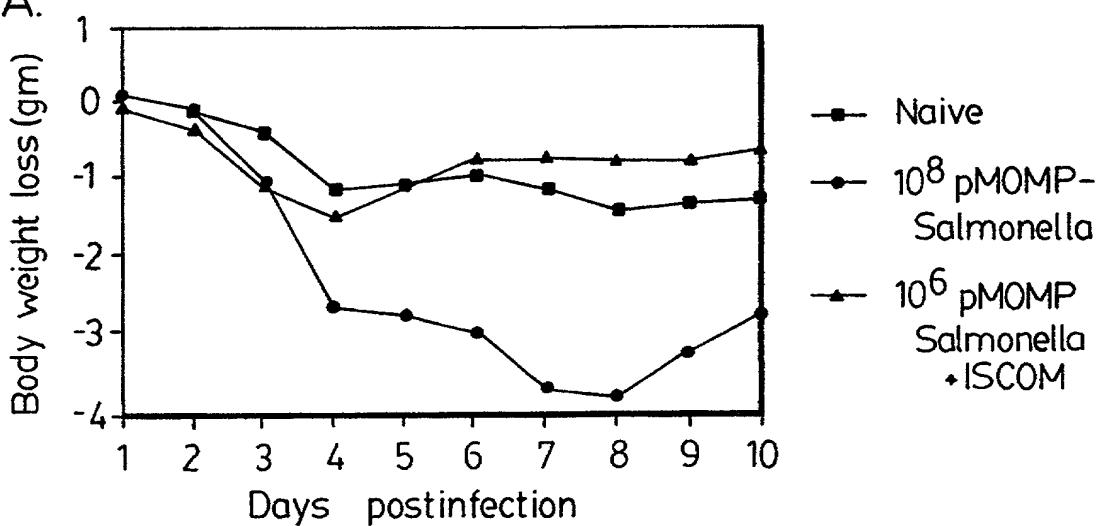


FIG.3B.

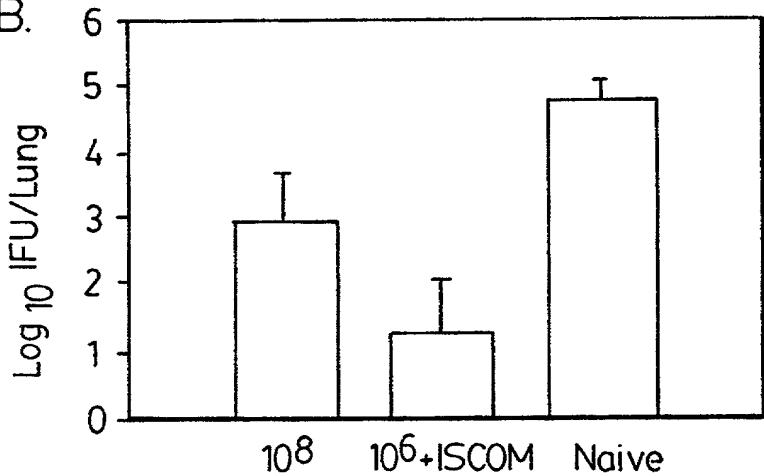
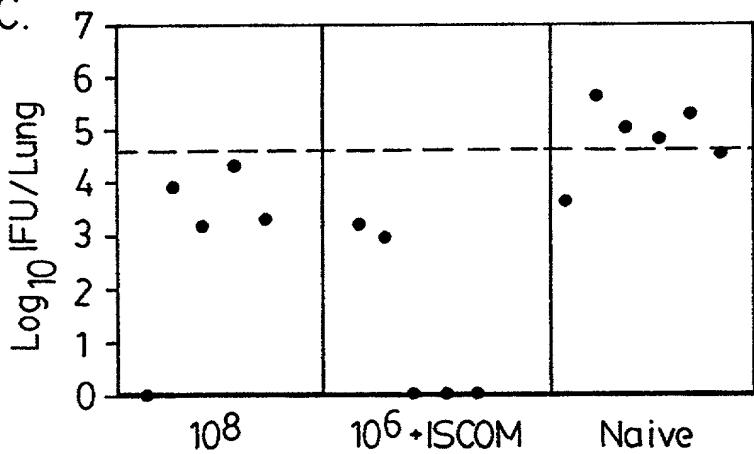


FIG.3C.



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FIG.4A.

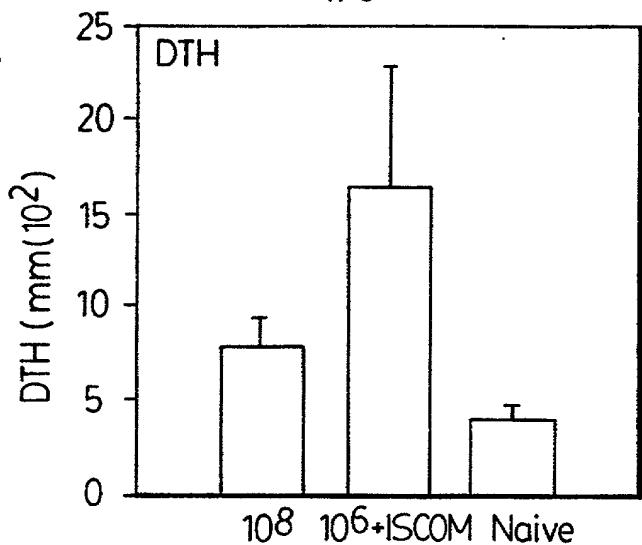


FIG.4B.

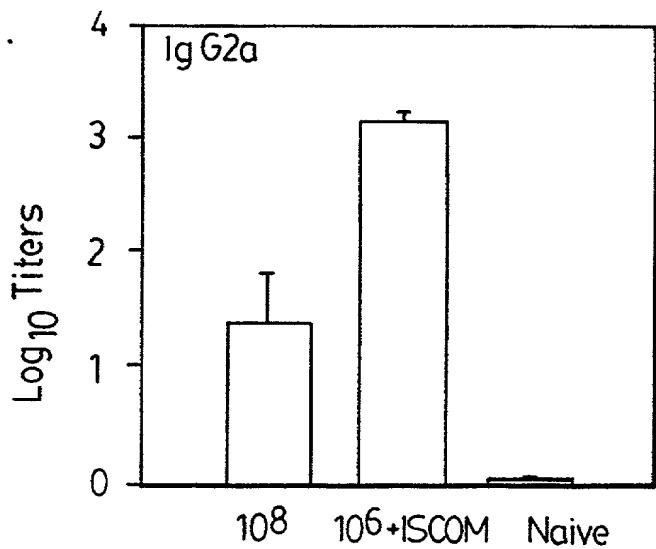
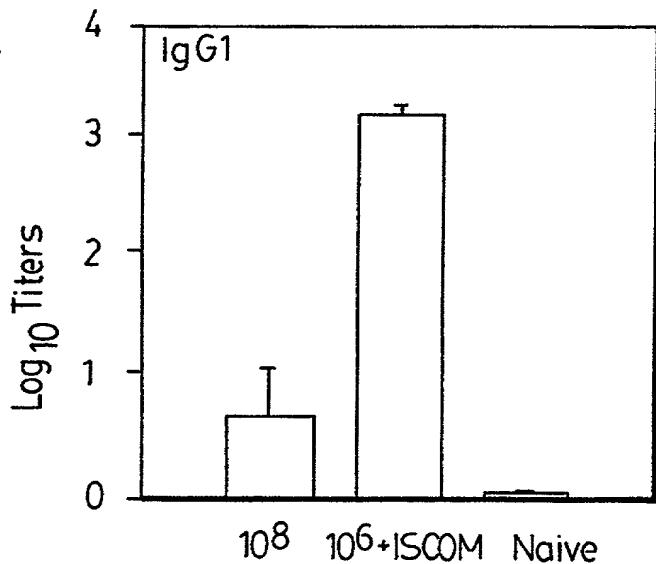
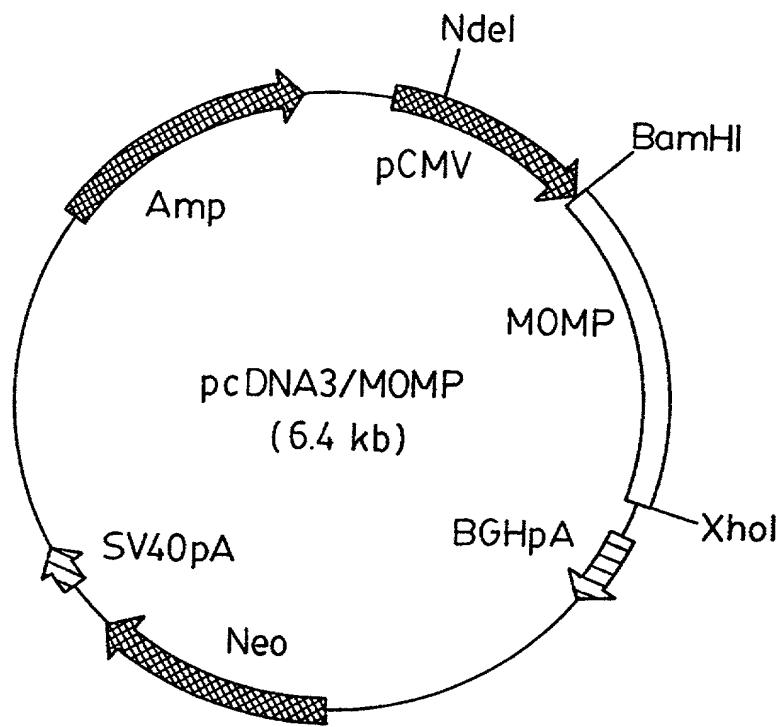


FIG.4C.



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FIG. 5.





Docket No.  
1038-1153 MIS:jb

# Declaration and Power of Attorney For Patent Application

## English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

### TWO-STEP IMMUNIZATION PROCEDURE AGAINST CHLAMYDIA INFECTION

the specification of which

(check one)

is attached hereto.

was filed on December 2, 1999 as United States Application No. or PCT International

Application Number PCT/CA99/01151

and was amended on \_\_\_\_\_

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Priority Not Claimed

(Number)	(Country)	(Day/Month/Year Filed)	<input type="checkbox"/>
(Number)	(Country)	(Day/Month/Year Filed)	<input type="checkbox"/>
(Number)	(Country)	(Day/Month/Year Filed)	<input type="checkbox"/>

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

60/110,855

(Application Serial No.)

December 4, 1998

(Filing Date)

(Application Serial No.)

(Filing Date)

(Application Serial No.)

(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)

(Filing Date)

(Status)  
(patented, pending, abandoned)

(Application Serial No.)

(Filing Date)

(Status)  
(patented, pending, abandoned)

(Application Serial No.)

(Filing Date)

(Status)  
(patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

**POWER OF ATTORNEY:** As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (*list name and registration number*)

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